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Research Note

Study Profiling of Artificial Liberica Civet Coffee with Stirred-tank Bioreactor Fermentation using *Bacillus subtilis*

Estudo do Perfil do Café Civet Liberica Artificial com Fermentação em Biorreator de Tanque Agitado usando Bacillus subtilis

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ABSTRACT

Jambi Province is one of the areas that is developing Liberica coffee plants. The unique thing about Liberica coffee is that it grows well in peatlands with high acidity levels. However, poor post-harvest processes and lack of treatment can reduce the quality of Liberica coffee. Fermentation is a metabolic process that uses sugar both aerobically and anaerobically. Fermentation of coffee can increase the cupping test value and influence its antioxidant activity. This research aims to analyze the effect of artificial liberica Civet Coffee in vitro using a stainless bioreactor on the sensory profile and proximate analysis. The research began with bioreactor design, fermentation process, and analysis of Liberica Coffee's fermentation results. The results showed that fermentation increased ash content, reduced fat content, and increased water content in vitro. Apart from that, the highest fermentation waste gas using a bioreactor is CO_2, reaching 400 ppm. The sensory profile of Liberian coffee fermented in vitro using a bioreactor increases the sensory value from 80.41 to 81.75 but is still lower than fermentation without a bioreactor, 86.30. Our findings revealed that fermentation can improve the sensory profile of Liberica coffee and is promising to increase the value of the Liberica coffee commodity.

KEYWORDS: *Bacillus subtilis.* Bioreactor. Fermentation. Liberica Coffee.

RESUMO

A Província de Jambi é uma das maiores produtoras de café da Indonésia. Uma característica notável do café Liberica é sua capacidade de crescer bem em solos de turfa com altos níveis de acidez, enquanto outros tipos de café não se adaptam adequadamente. No entanto, processos pós-colheita deficientes e tratamentos insuficientes têm contribuído para a redução da qualidade do café Liberica. A fermentação é um processo metabólico que utiliza nutrientes químicos, como açúcares, aminoácidos e até minerais, em reações tanto aeróbicas quanto anaeróbicas. A fermentação do café é amplamente reconhecida por aumentar o valor do perfil sensorial e melhorar sua atividade antioxidante. Esta pesquisa tem como objetivo caracterizar o efeito da fermentação artificial do café Civeta Liberica **in vitro** utilizando um biorreator de aço inoxidável no perfil sensorial e na análise proximal. O estudo começou com o design do biorreator, o processo de fermentação e a análise dos resultados da fermentação do café Liberica. Os resultados mostraram que a fermentação aumentou o teor de cinzas e de água, enquanto reduziu o teor de gordura, proteína e carboidratos. O gás residual de fermentação mais elevado utilizando o biorreator foi o CO_2, atingindo 400 ppm. O perfil sensorial do café Liberica submetido à fermentação **in vitro** com biorreator demonstrou uma melhoria nos escores sensoriais, passando de 80,41 para 81,75. No entanto, esse valor ainda é inferior ao alcançado por métodos tradicionais de fermentação, que chegaram a 86,30. Nossos achados revelaram que a fermentação em biorreator pode melhorar o perfil sensorial do café Liberica e mostra-se promissora para aumentar o valor da commodity de café Liberica. A otimização do processo de fermentação utilizando biorreatores precisa ser realizada para obter resultados ainda melhores.

PALAVRAS-CHAVE: *Bacillus subtilis*. Biorreator. Fermentação. Café Liberica.

INTRODUCTION

Indonesia is currently ranked fourth in the world's top coffee producers based on October 2019 International Coffee Organization (ICO) data (ICO 2019). Origin coffee is reported to have different taste characteristics and is now marketed globally as single-origin coffee with a unique taste and metabolic profile (MOLDVAER 2014, PEREIRA et al. 2019). However, the quality of Indonesian coffee remains inconsistent, largely influenced by variations in environmental conditions during the post-harvest process (PEREIRA et al. 2014, DE BRUYN et al. 2017). Apart from that, the coffee commodities developed are still limited to Robusta and Arabica, while Liberica is still very minimal (LATIEF et al. 2020).

Jambi Province is one of the largest coffee-producing provinces in Indonesia. Three types of coffee are cultivated, including arabica, robusta (Kerinci Mountains area), and Liberica (swamp area) (MAWARDHI & SETIADI 2018, SUHARYONO & BUSYRA 2019). One effort to increase the added value of coffee is by preparing coffee products using fermentation technology (WILUJENG & WIKANDARI 2013).The coffee bean fermentation process breaks down complex compounds in coffee beans into simpler compounds involving several microorganisms. The fermentation process affects the quality and taste of coffee in coffee beans (PANGGABEAN 2011). One well-known example of fermented coffee is Luwak coffee, which undergoes in vivo fermentation in the digestive tract of the civet (*Paradoxurus hermaphroditus*) after consuming ripe coffee cherries. The process involves enzymatic activity in the civet's stomach, producing beans with distinct chemical and sensory characteristics (JUMHAWAN et al. 2013). The coffee fruit then undergoes a fermentation process by various digestive enzymes. It is excreted with the civet droppings in the form of coffee beans which are still covered in horny skin. Penetration of stomach acid and digestive enzymes during fermentation affects the chemical compounds in coffee beans and causes the coffee beans to become porous and more brittle. The natural fermentation process in the intestine by lactic acid also affects the taste of coffee (JUMHAWAN et al. 2015b).

Its unique taste and unique origins have caused Luwak coffee to become increasingly sought after by local and world coffee lovers, increasing demand for this product at a phenomenal price. However, mongoose cultivation has several weaknesses, including expensive production costs, and can threaten the sustainability of wild mongooses in nature (JUMHAWAN et al. 2015b). CARDER et al. (2016) highlighted the exploitation practices that pose a significant threat to the survival of civets, particularly in the context of kopi luwak production. This industry frequently relies on civets being confined in small cages with wire flooring, devoid of access to their natural habitat. Such conditions often result in inadequate nutrition, limited access to clean water, and restricted mobility, all of which contribute to poor physical health and welfare (WHELAN et al., 2023). Therefore, an alternative method of making environmentally friendly civet coffee is needed without reducing the quality of the civet coffee produced.

One alternative is producing artificial civets using civet digestive tract microbes, which can produce unique fermented coffee with a distinctive taste and aroma (DEWI et al. 2012). Fermentation using lactic acid bacteria from civet digestion has been

developed in several studies. GUNTORO (2010) carried out coffee fermentation using Lactobacillus sp bacteria and Bifidobacterium sp., which were isolated from the digestion of civet animals and produced quite high cupping test values, namely 8.21-8.25. Previous research carried out by TARIGAN et al. (2024a) developed a Liberica coffee fermentation biotechnology using *Alcaligenes* sp. and *Exiguobacterium indicum*, resulting in improved coffee sensory, with the cupping test value increasing up to > 8.0. The same research also increased total phenolics, flavonoids, and antioxidant activity. Fermentation also increases the bioactive compound profile of Liberica Coffee. Another research conducted by USMAN et al. (2015) isolated bacteria suspected to be lactic acid bacteria from civet feces and carried out coffee fermentation using this isolate. The results showed a decrease in caffeine and pH in fermented coffee.

Optimization and scale-up of coffee fermentation research results must be done by developing bioreactors and analyzing their effects. In fermentation, we usually use Stainless Stirred-Tank Bioreactor analysis to determine the product's levels. A bioreactor is a piece of equipment or system that can provide a biological environment to support biochemical reactions from raw materials to the desired materials. Biochemical responses in bioreactors involve organisms or active biochemical components (enzymes) originating from certain microorganisms, both aerobically and anaerobically. Fermentation in a bioreactor can improve the quality of coffee, standardize the fermentation process, and produce exceptional coffee. Sensory perception shows that inoculated yeast modifies flavor attributes, improves quality, and increases coffee SCA scores (MARTINEZ et al. 2021). Other research reported that the stainless-tank bioreactor (STR) fermentation process led to the production of coffee beans with a more decadent aroma and beverage composition and significant improvements in the sensory analysis of coffee drinks compared to those produced from conventional processes. This fermentation model can carry out controlled bean fermentation to supply the coffee industry with homogeneous, high-quality coffee beans (CARVALHO NETO et al. 2017, 2018). Optimizing STR using a stirrer will increase the homogeneity of the fermentation process. It is the industry's standard reactor and is widely used, especially in biotechnology (KADIC & HEINDEL 2010). This research aims to design a Stainless Stirred-Tank Bioreactor for Fermenting Liberica Coffee using Lactobacillus and its effect on the sensory, proximate, and exhaust gas profiles.

MATERIAL AND INSTRUMENTATIONS

The material for this research is Liberika Coffee, obtained from East Tanjung Jabung, Jambi Province. The bacterial strain used is *Bacillus subtilis*. The ingredients needed are MRS NA, Aquades, MRS Broth, NaCl 0.9%, Methanol, Acetone, Methoxyamine Hydrochloride, MSTFA, Gallic Acid, Folin-Ciocalteu, Na_2CO_3, $AlCl_3$, Quercetin, Na-CH_3COO, NaOH, Phosphate Buffer, $NaSO_4$, $CuSO_4$, H_2SO_4, HCl, Phenophthalein Indicator, KI, Starch Indicator, n-Hexane, and DPPH (Sigma-Aldrich, Singapura Ltd.). The equipment used in this research was Erlenmeyer 100 mL, hot plate, stirring rod, magnetic stirrer, refrigerator, aluminum foil, autoclave, petri dish, incubator, Erlenmeyer 500 mL, tube needle, volume pipette, test tube, colony counter,

glass bottle, SAS coffee roaster, micropipette, centrifuge, Eppendorf tube, vortex, 5 mL volumetric flask, separating funnel, beaker, funnel, 250 mL Erlenmeyer, water bath, 0.45 µm PTFE filter paper, 10-micron vial, porcelain cup, oven, desiccator, scales, Kjehdahl flask, set of distillation tools, set of titration tools, set of Soxhlet tools, dropper pipette, water bath, furnace, and FT-IR (Themo-Scientific).

METHODS

Design Stirred-tank Bioreactor Stainless

The bioreactor is designed with a size of 0.3 m^3 and manufactured using stainless steel to ensure satisfactory hygiene conditions for the production results. The design and concept refer to previous research (MARTINEZ et al. 2021). The stainless-steel tank is designed as a cylinder with a height of 900 mm and a diameter of 650 nm. At the bottom, it consists of a cone-shaped funnel with a slope of 250, allowing fluid from exudation to be removed easily. The tank also has a butterfly valve manufactured in stainless steel with a diameter of 4″. This valve allows total or partial opening and release of the coffee after fermentation and the exudates produced during fermentation, allowing them to dry more quickly. Temperature was measured with three identical thermometers at the beginning and continuously until the end of fermentation. It is an essential parameter for monitoring microbial activity and is considered a final indicator of fermentation.

Inoculum Preparation

The bacteria used were the probiotic bacteria groups *Bacillus subtilis.* The bacterial inoculum was activated in Luria Bertani (LB) medium (1% Tryptone, 0.5% NaCl, and 0.5% Yeast Extract) at 37 °C with a stirring speed of 125 rpm for 16–18 h. The Optical Density value of bacterial isolates used is 0.3-0.8, with a 107 CFU/mL density. The culture is then used as an inoculum for coffee fermentation (ADITIAWATI et al. 2020).

Fermentation and Roasting

Coffee is sterilized using gamma irradiation at a dose of 2.5 kGy. Coffee is added to the bioreactor, which already contains inoculum at a ratio of 1:1. Fermentation was carried out for 12 hr at 37 °C (HAILE & KANG 2019b, ADITIAWATI et al. 2020). During Fermentation, the coffee is mechanically homogenized every 4 hr to ferment uniformly. After Fermentation, the coffee is transferred to a platform and dried in the sun until it reaches a moisture content of 11%. During Fermentation, the pH value and exhaust gas profile are analyzed. Fermented coffee beans are rinsed, dried in the sun, and dried in an oven at 50–60 °C for three days. The dried coffee beans are then roasted using a SAS coffee roaster (200 °C for 2.5-4 min). Roasting was conduted at three types of temperatures; light (170 ^{0}C), medium (190 ^{0}C), and dark (210 ^{0}C) for 12 min.

Cupping Test Sensori and Proximate Analysis

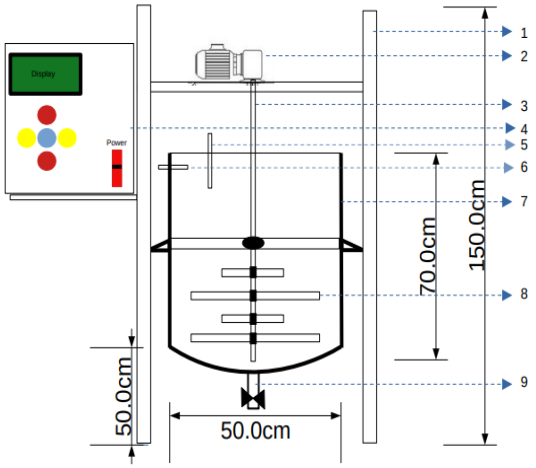
The fermented coffee sample that has been roasted is then ground to a size of 20-mesh to form a powder brewed with 150 mL of hot water (94-96 °C) for the Cupping Test. Assessment of coffee sensory attributes is carried out by experts, including aroma attributes, flavor, aftertaste, acidity, body, balance, uniformity, sweetness, a clean cup, and overall. Panelists rated each sensory detail with a score of 6.00 to 6.75(good), 7.00 to 7.75 (very good), 8.00 to 8.75 (excellent), and 9.00 to 10.00

(outstanding). The final score is obtained by summing the score of each attribute (KITZBERGER et al. 2020)

RESULTS AND DISCUSSIONS

Bioreactor

The bioreactor is designed to be 0.15m^3 in size, and stainless-steel material is used for the bioreactor tube to ensure the hygiene of the fermentation results. The bioreactor tube has a tube height of 700.0mm and a tube diameter of 500.0mm. The bioreactor tube support is made using iron material with dimensions of 20.0x20.0mm; the height of the tube is 150.0cm, and the width is 60.0cm. At the bottom, it consists of a cone-shaped funnel with a slope of 150.0mm and is equipped with a tap, making removing the liquid from the bioreactor exudation easy. A DC motor is used with a max-rotation speed of 88 rpm to drive the stirred rod, which can be adjusted from the electronic control unit. Besides controlling the motor rotation speed, the electronic control unit has temperature and gas-measuring instruments. The two measuring instruments are installed in the reactor tube, as seen in Figure 1.



Bioreactor Specifications	
Height	: 700mm
Diameter	: 500mm
Capacity	: 0.15m^3
Materials	: Stainless steel
Motor	: Single-phase
Power Supply	: 5 VDC *for* electronic control
Motor Speed	: 10 - 88 rpm

Figure 1. Bioreactor Design.

Fermentation and Profile Sensory

In this study, wet processing of coffee was carried out. The damp coffee processing process includes pulping, fermentation, drying, and grinding. Improper processing can result in over-fermented coffee beans. Coffee fermentation procedure by soaking liberica coffee beans into dark glass bottles with lids containing MRS Borth media with a total volume of 500 mL. The bacterial inoculum was transferred as much as 64.5 x 10^{-7} CFU/mL into a glass vial containing fermentation media. In the fermentation process, the mucus that envelops the coffee beans is used by bacteria as a source of nutrients to grow and replicate.

The fermentation process involving bacteria induces significant biochemical changes in the biosynthesis of chemical compounds that serve as precursors to flavor, including a reduction in the level of reducing sugars. This study utilized *Bacillus subtilis* (BC), which led to changes in the pH of the fermentation liquid. As shown in Figure 2, the initial acidity (pH) of the solution was 6. After 12 hr of fermentation, the pH remained

unchanged. However, after 24 hr of fermentation, the pH decreased to 5, and further declined to 4 after 36 hr of fermentation.

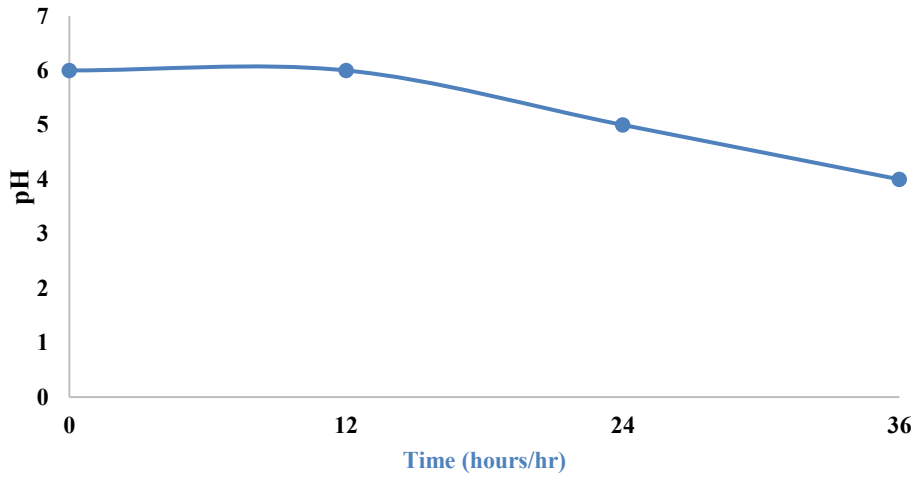


Figure 2. pH changes that occur during coffee fermentation.

The fermentation process is influenced by microbial activity, which lowers coffee's caffeine and pH levels. Decreased pH levels are affected by the accumulation of organic acids and an increase in the number of H^+ protons due to bacterial metabolism due to the breakdown of amino acids. According to AFRILIANA *et al* (2018), the duration of fermentation is directly proportional to microbial growth, resulting in an increased utilization of substrates for microbial metabolism. This is further supported by De CARVALHO *et al* (2017), who stated that microbial growth is accompanied by the regular consumption of sugars (glucose and fructose) and their conversion into organic acids. The relationship between organic acid concentration and caffeine content is inversely proportional, where an increase in acid concentration corresponds with a decrease in caffeine levels as fermentation time progresses. This suggests that microbial activity is capable of degrading caffeine (HATININGSIH et al., 2018). After that, the roasting process of original Liberica coffee and Fermented Liberica coffee is carried out with a roasting machine that rotates continuously. In this study, roasting was carried out at a temperature of 203 °C (medium roast), which showed the color of the coffee beans produced was blackish brown and the aroma of thick coffee. In the roasting process, it is possible to change chemistry, which can be seen from the change in the color of coffee beans from bluish-green to blackish-brown.

Experts conduct sensory testing to determine the main sensory components following the rules of the Specialty Coffee Association (SCA), which has been considered globally as a method of sensory assessment of coffee drinks. The cupping procedure begins by grinding Original Liberica coffee beans. Fermented Liberica coffee was roasted and ground using a Latina grinder to a coarse level of fineness, then brewed using the Tobruk technique with hot water at 92–96 °C. Cupping was conducted to evaluate the sensory characteristics of the coffee, including aroma (dry fragrance), flavor (distinctive coffee taste), body (viscosity), acidity (perceived sourness), aftertaste (lingering impression), sweetness, balance (harmony of flavor and aroma), clean cup (clarity), uniformity (consistency), and overall quality.

The assessment range of each quality attribute is 1-10, where the total value for sensory testing is the result of adding the value of each quality attribute. The results of the assessment conducted by selected experts are presented in Table 1.

Table 1. Profile of cupping test score.

Parameters	Samples						
	H1	H2	H3	I1	I2	I3	BC
Overall	6.50	7.25	7.25	6.00	7.00	7.00	8.50
Aroma	7.00	7.00	6.75	7.50	7.00	6.50	8.25
Flavor	6.50	7.00	7.75	6.50	6.75	7.25	8.00
Aftertaste	6.75	7.75	7.75	6.50	6.25	7.25	8.00
Acidity	6.00	7.75	7.75	6.00	7.00	7.00	7.70
Body	6.50	7.25	7.25	6.75	7.25	7.00	7.90
Balance	6.50	7.00	7.25	6.50	7.50	7.00	7.90
Uniformity	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Clean cup	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sweetness	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Final Score	75.00 ± 0.015	81.75± 0.025	78.33± 0.015	75.75± 0.045	80.41± 0.035	83.25± 0.05	86.30 ± 0.104

Sample codes:
- H1: Fermentation BC w Bioreactor (Dark Roasting)
- H2: Fermentation BC w Bioreactor (Medium Roasting)
- H3: Fermentation BC w Bioreactor (Light Roasting)
- BC : Fermentation BC w.o Bioreactor (Medium Roasting)
- I1: Liberica Luwak Coffee (Dark Roasting)
- I2: Liberica Luwak Coffee (Dark Roasting)
- I3: Liberica Luwak Coffee (Dark Roasting)

Exhaust Gases

The exhaust gas produced in the Liberica Coffee fermentation process depends on the length of time needed during the process. Furthermore, the roasting temperature affects the magnitude of the output gas concentration. The effect of the output gas on time and temperature can be seen in Table 2 and Figure 3.

Table 2. Exhaust Gas Profile During Fermentation.

Time (min)	Gases					
	CO	Alcohol	CO_2	Toluene	Amino	Acetone
5	14.83	3.86	407.45	1.75	9.95	1.45
10	28.33	6.51	411.93	3.08	14.94	2.52
15	35.33	7.79	414.01	3.74	17.16	3.05
30	27.46	6.35	411.66	3	14.65	2.46
45	5.22	1.66	403.49	0.7	5.16	0.59
60	1.62	0.65	401.49	0.25	2.48	0.22
75	0.66	0.31	400.77	0.11	1.41	0.1
90	0.3	0.17	400.44	0.06	0.87	0.05
105	0,18	0.11	400,31	0.04	0.63	0.03
120	0,13	0.09	400,24	0.03	0.51	0.03
135	0.10	0.07	400.19	0.02	0.42	0.02
150	0.08	0.06	400.16	0.02	0.37	0.02
165	0.07	0.05	400.15	0.02	0.34	0.01

Time (min)	Gases					
	CO	Alcohol	CO_2	Toluene	Amino	Acetone
180	0.06	0.04	400.13	0.01	0.31	0.01
195	0.06	0.04	400.13	0.01	0.3	0.01
210	0.05	0.04	400.12	0.01	0.28	0.01

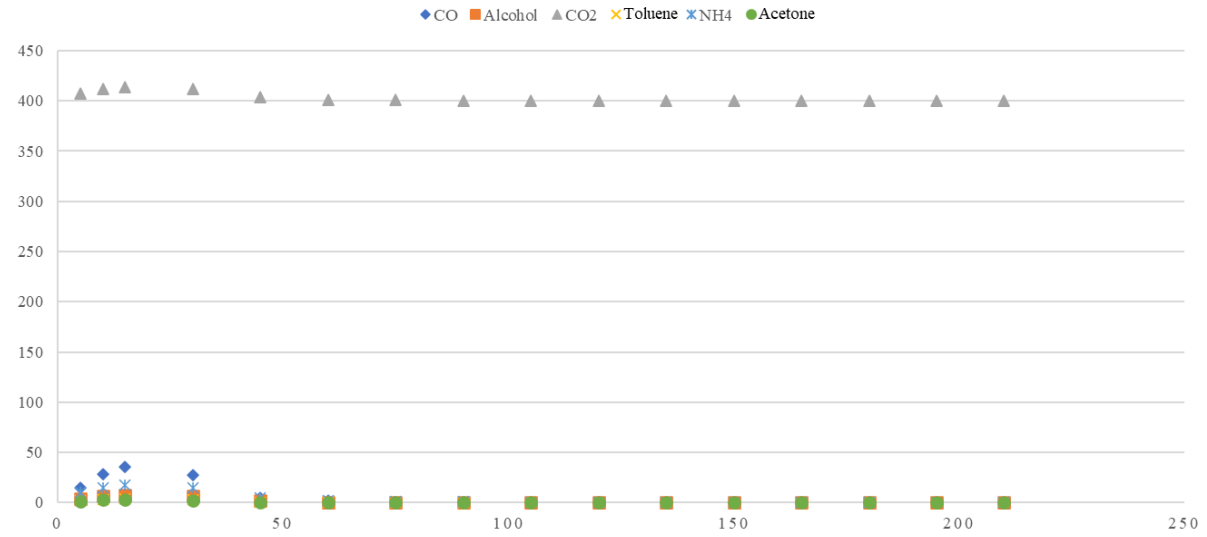


Figure 3. Exhaust Gas Profile During Fermentation

The CO gas concentration is high in the first 30 min in the 14 – 35 ppm range. In the first 30 min, Amino gas was detected in the 1.41 – 17.16 ppm range. In fermentation, microbial activity aerobically converts saccharide groups into water and carbon dioxide, while anaerobically converts saccharides into alcohol and carbon dioxide groups. If excess glucose is present, ethanol may be produced through reaction 2, even under aerobic conditions. Finally, an aerobic state of the fermentation exists where ethanol is consumed, making water and carbon dioxide through reaction 3. Still, this state is only active in the absence of glucose (GEINITZ et al. 2020).

Reaction 1: $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2$

Reaction 2: $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2\ CO_2$

Reaction 3: $2C_2H_5OH + 3\ O_2 \rightarrow 3\ H_2O + 2\ CO_2$

Profiling IR Finger-Print

This research used FTIR (Fourier Transform Infrared) to measure using reflectance sample handling techniques, and analysis was recorded in transmittance with a wave number range of 4000-400 cm^{-1}. In the research, FT-IR analysis aimed to determine the comparative effect of fermentation time and coffee roasting temperature on the compounds produced. The differences can be seen from Figure 4 and Table 3.

The analysis results show that the IR spectra of coffee samples with a roasting temperature of 170 ℃ show no differences in the spectral character of each treatment; the spectra of non-fermented coffee, 24 and 48-hour fermentation are similar. However, there is a shift in the resulting peak. According to SIMATUPANG et al. (2023), in the range 3743-3302 cm^{-1}, this area is typical of the OH functional group, which is closely related to phenolic compounds in coffee, the area 3409-3322 cm^{-1} is related to the N-H group of characteristic secondary amines caffeine compound, the

region 2853-2925 cm^{-1} corresponds to the C-H functional group of aliphatic CH_2 in fatty acids. SAHACHAIRUNGRUENG et al. (2022) stated that the lipids in coffee consist of triglycerides, sterols, fatty acids, and the pentacyclic diterpene compounds cafestol and kahweol. The region 1760-1690 cm-1 C=O includes the amide carbonyl group; this carbonyl refers to aldehydes and carboxylic acids.

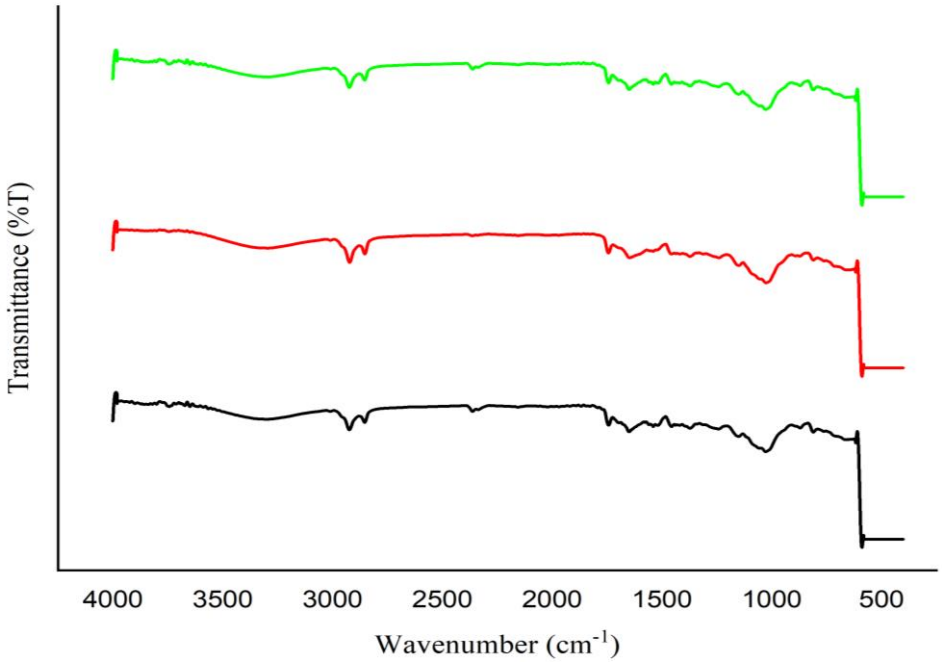


Figure 4. IR-Spectrum Liberica Coffee. Red lines (Fermentation with Bioreactor), Green lines (Liberica luwak Coffee), and Black lines (Non-fermentation)

Table 3. IR spectrum coffee liberica.

Functional Groups	Wavelenght (cm^{-1})		
	Non-fermentation	Liberica Luwak Coffee	Fermentation with Bioreactor
O-H	3743.74	3322.84	3330.06
N-H	3406.73	3349.84	3344.78
C-H	2922.67	2921.53	2921.53
C=O	1741.94	1741.17	1744.85
C=C	1647.44	1646.64	1650.33
N-C-C-O	1511.65	1511.65	1511.65
C-O	1152.29	1147.007	1150.69
C-N	1205.81	1301.75	1312.88
R-OH	1040.49	1055.21	1033.12
C-O	809.02	810.63	810.63
C-H	738.65	738.65	738.65

Aldehydes are one of the compounds that influence the final aroma when coffee is roasted. Long-chain aldehydes usually have a pleasant and soft fruity and floral aroma. The area 1665-1635 cm-1 of the C=C functional group on the phenyl ring is the absorption peak of the functional group found in antioxidant compounds, trigonelline, and chlorogenic acid (LEE et al. 2016). The region 1522-1507 cm-1 with the N-C-C-O group is included in the amine group, which is found in amino acid compounds (WANG & LIM 2012).

The region 1176-1106 cm^{-1} identifies the C-O functional group in alcohols related to sugar and chlorogenic acid. The 1360-1180 cm^{-1} area contains the C-N functional group in amines, related to amino acids as well as CH_3 and CH_2 bonds, aromatic chains, and primary and secondary amines; this group is a characteristic of coffee's caffeine compounds (FIORESI et al. 2021). The region 1064-1029 cm^{-1} identifies the R-OH group associated with chlorogenic acid. The 804-817 cm^{-1} region identifies the C-O functional group associated with chlorogenic acid compounds. There is a prevalence of carboxylic acids, amines, amino acids, and proteins that contribute to the characteristics of compounds in coffee (SIMATUPANG et al. 2023). The 745-735 cm^{-1} region identifies the C-H functional group related to aromatic compounds and alkenes (SARAGIH et al. 2021).

Proximate Analysis

Water Content: Water content is one of the physical properties of agricultural materials that can influence the quality of coffee beans, which is related to the length of storage time. In this study, water content analysis was carried out by comparing non-fermented (O), Liberica luwak coffee (I), and fermented coffee using a bioreactor (H). Water content analysis in this study is based on SNI-01-3542-2004.

The results show that fermentation using bioreactors and civets influences the resulting analysis. The % water content results in the table above are by the maximum limit of SNI-01-3542-2004, about 7%. According to FAUZI et al. (2017), the decrease in coffee water content is due to the longer fermentation time due to the increase in temperature and microbial activity, which causes enzyme activity to be more active and the pulp to be watery. Heat will affect the destruction of the pulp, and the pores of the seeds will open, so the water content will evaporate more quickly during drying. According to BARUS (2019), increasing microbial activity can also increase the decomposition of various compounds in coffee beans. Including the breakdown of protein and carbohydrate compounds into simpler compounds. In fermentation, protein compounds are broken down into peptides and amino acids through a hydrolysis process with the help of protease enzymes.

Furthermore, the hydrolysis process by microbial carbohydrates is broken down into reducing sugars, ethanol, and organic acids. This process always requires water. The longer the fermentation process is carried out, the water content should decrease. By previous research, the 48-hour fermentation process has a water content of 2.36% and non-fermentation of 3.03%, because this decrease is directly proportional to the fermentation time; the longer the fermentation, the greater the rate of decrease in the water content of the coffee, the more it decreases by SNI standards (TAWALI et al. 2018).

Ash Content: The study used a gravimetric method to analyze the ash content of fermented Liberica coffee. The high or low ash levels in coffee beans depend on the mineral content, including potassium, calcium, magnesium, and non-metallic minerals, including phosphorus and sulfur (PAMUNGKAS et al. 2021). These minerals are obtained from nutrients absorbed during growth. The ash content in this study was based on the SNI standard -01-2907-2008. The analysis results show that the fermentation length and roasting temperature differences in Liberica coffee processing

directly influence the ash content. It can be seen that the range of ash content obtained with a fermentation period of 24 hr is 2.31-3.16%. During fermentation for 48 hr, the ash content range is 1.45-2.99%; the longer the fermentation is carried out, the lower the ash content produced. According to (ZAINUDDIN & TOMINA 2021), the ash content decreases as coffee bean fermentation takes longer due to the large number of water- and fat-soluble minerals that come out together during the processing process, namely the drying and pressing processes. The process related to the dissolution of compounds in coffee beans is carried out when fermentation is carried out.

Table 4. Proximate Profile.

Samples	Water content	Ash content	Fat content	Protein content	Carbohydrates content
Non-Fermentation (O)	4.30%	3.55%	10.17%	2.09%	71.24%
Luwak Liberica (I)	4.46%	4.81%	9.83%	0.74%	22.38%
Fermentation with Bioreactor (H)	5.57%	4.47%	9.28%	0.78%	21.25%

Roasting temperature also influences the ash content produced. The higher the temperature and longer the roasting time, the lower the ash content. (PAMUNGKAS et al. 2021) stated that reducing ash content with high roasting temperatures does not have a natural effect because minerals have properties that are not easily damaged by processing. However, processing can cause mineral losses of 3% in food sources so that ash content can be reduced by more than 0.04%. Meanwhile, (YUHANDINI et al. 2008) stated that the quality of the coffee causes differences in coffee ash content. Good quality coffee will be cleaner and have a higher mineral content, so the ash content produced will be higher. The ash content is according to the SNI -01-2907-2008 standard for ground coffee, which states that the ash content of ground coffee is no more than 5%. According to (MULLER et al. 2013), if the coffee ash content is more than 5%, it contains foreign substances (impurities). The ash content obtained in this study is by SNI Standards.

Fat Content: This research also tested the fat in fermented Liberica coffee by comparing the types of fermented coffee using the soxhletation method with petroleum benzene as a non-polar organic solvent. The results of the fat content analysis in this study can be seen in Table 4. The analysis results show that fermentation influences the % fat produced. The fat content range for non-fermented coffee is 9.93-10.53%. Meanwhile, fermented coffee has a 7-8% fat content. Fermentation plays a role in influencing the fat content produced because the longer fermentation is carried out, the fat content will decrease. According to SILABAN et al. (2023), lipid content decreases during fermentation. One reason is that the fat bonds are damaged during the fermentation process because the fat content undergoes an oxidation process, which causes the double bonds to be damaged. Meanwhile, HANIFAH & KURNIAWATI (2013) stated that the enzymes in coffee pulp are hydrolyzed by water,

so that the lipase enzyme, with the help of bacteria, breaks down fat into fatty acids, which causes a decrease in fat content, as the fermentation process takes longer.

According to KIYAT et al. (2019), during fermentation, bacteria activate several enzymes, such as lipase, which converts fat and free fatty acids into butyric acid. The high level of butyric acid produced affects the sensory quality of coffee. Apart from that, roasting temperature also affects the fat content produced. SILABAN et al. (2023) stated that the roasting process increases fat content due to decreased water content.

According to MARDIANA et al. (2021), the roasting process causes changes in the composition of chemical compounds in coffee beans caused by the Maillard reaction and degradation of chemical compounds during roasting. One of the compounds that changes is fat. Fat is one of the chemical components in coffee that forms the taste of coffee and can produce beneficial effects. The fat content in coffee is mainly found in coffee oil, which is in the endosperm of green coffee beans, and a small part in the coffee wax layer, which is in the outer layer of coffee beans. This layer has 5-hydroxytryptamine fatty acids from palmitic, arachidic, behenic, and lignoceric acids. However, the fat content values obtained did not increase significantly. According to PAMUNGKAS et al. (2021), the oil's fat content is stable enough to heat and will evaporate slightly, which is thought to be the aroma-forming compound in coffee.

Protein content: The highest protein content presented in Table 4 is found in non-fermented coffee samples at 2.09%, followed by fermented coffee using a bioreactor at 0.78%, and the lowest protein content in civet coffee at 0.74%. Referring to previous research, non-fermented coffee samples had higher protein content compared to fermented coffee samples, at 17.61% and 14.74%, respectively by (TARIGAN et al., 2024b). Similar to carbohydrates and fats, proteins are complex organic compounds with high molecular weight. Fermentation can lead to a reduction in protein content in coffee beans. This decrease can be attributed to the increased decomposition of various compounds in the beans, driven by enhanced microbial activity, which results in competition among bacteria for proteins as nutrients to produce metabolites. During fermentation, some proteins are utilized as a nitrogen source for microbial growth, leading to a decline in protein levels.

In fermentation, proteins are broken down into peptides and amino acids through hydrolysis facilitated by protease enzymes. During this process, the protein structure is degraded and broken into smaller and simpler components. Consequently, this results in a reduction in the protein content of coffee.

Carbohydrates content: The carbohydrate components in coffee beans are essential for aroma development, particularly through the caramelization of low molecular weight molecules and the Maillard reaction with amino acids. The carbohydrate content in fermented coffee is lower compared to unprocessed coffee (LATIEF et al., 2023). The data presented in Table 4 indicates that coffee fermented using a bioreactor has the lowest carbohydrate content at 21.25%, followed by civet coffee at 22.38%, and the highest carbohydrate content in non-fermented coffee at 72.24%.

Referring to previous research conducted by TARIGAN et al. (2024), the carbohydrate content of fermented Liberica coffee using Bacillus subtilis bacteria was

3.67%, which represents a decrease from the original coffee's carbohydrate level of 5.96%. The bacteria present during fermentation require energy obtained from the breakdown of organic and inorganic compounds, most of which is provided by carbohydrates. The utilization of carbohydrates as an energy source by bacteria for growth contributes to the reduction in carbohydrate levels. During fermentation, carbohydrates are broken down by bacteria into simpler compounds such as glucose in the fermented coffee. Furthermore, microbes during fermentation require energy from the decomposition of organic or inorganic compounds, which is primarily supplied by carbohydrates (SHARMA et al., 2020; LATIEF et al., 2023).

CONCLUSION

This study demonstrates that the fermentation of Liberica coffee using Bacillus subtilis and varying fermentation durations significantly influences biochemical parameters such as pH reduction, total lactic acid bacteria growth, ash content, and fat composition. The findings provide deeper insight into the role of microbial activity, particularly lactic acid-producing bacteria, in developing flavor precursors and modifying the chemical profile of coffee. The research advances current knowledge by optimizing fermentation conditions to improve coffee quality through controlled microbial intervention and bioreactor application. The integration of a custom-designed stainless-steel bioreactor equipped with adjustable stirring and real-time temperature and gas monitoring offers a practical tool for standardizing fermentation processes at a semi-industrial scale. Future studies should explore the sensory evaluation of fermented coffee, the profiling of volatile compounds, and the interaction of other microbial strains to further enhance flavor development. Additionally, ongoing work involves scaling the process and testing its application on different coffee varieties to evaluate its broader applicability in specialty coffee production.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis**, SS, ILT and ML**; software and validation**, SS, IIR and ILT;** investigation**, IIR, ILT and MFA**; resources and data curation**, IIR, ILT and MFA**; writing-original draft preparation**, ILT and IIR**; writing-review and editing**, SS, ILT and IIR**; visualization**, ILT, ML and IIR**; supervision, **S, ML and ILT**; project administration, S**S, ILT and ML**; funding acquisition, **SS, ILT and ML**. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

ADITIAWATI A et al. 2020. GC/MS-based metabolic profiling for the evaluation of solid-state fermentation to improve quality of Arabica coffee beans. Metabolomics 16: 57.

AFRILIANA A et al. 2018. Fermented Technology of Robusta Coffee Beans (Canephora Coffee) With Kefir Milk to Produce Specialty Coffee. Advances in Engineering Research 172: 302–309.

BARUS WBJ. 2019. Pengaruh lama fermentasi dan lama pengeringan terhadap mutu bubuk kopi. Wahana Inovasi 8(2): 111–115.

CARDER G ET AL. 2016. The animal welfare implications of civet coffee tourism in Bali. Universities Federation for Animal Welfare 25: 199-205.

CARVALHO NETO D et al. 2017. Yeast diversity and physicochemical characteristics associated with coffee bean fermentation from the Brazilian cerrado mineiro region. Fermentation 3: 11. doi: 10.3390/fermentation3010011.

CARVALHO NETO DP et al. 2018. Efficient coffee beans mucilage layer removal using lactic acid fermentation in a stirred-tank bioreactor: Kinetic, metabolic and sensorial studies. Food Bioscience 26: 80–87.

DE BRUYN F et al. 2017. Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green coffee bean production. Applied and Environmental Microbiology 83: e02398–e2416.

DEWI KH et al. 2012. Kajian Komposisi Bahan Baku Kopi Ginseng Lau dan Kopi Ginseng Panax. Proseding Seminar Nasional Tumbuhan Obat Indonesia.Universitas A. Yani. Bandung.

GEINITZ B et al. 2020. Gas Fermentation Expands the Scope of a ProcessNetwork for Material Conversion: Review Article. Chemie Ingenieur Technik 92: 1665–1679.

GUNTORO S. 2010. Proses Memproduksi Kopi Luwak Probiotik. Proposal Paten. Balai Pengkajian Teknologi Pertanian (BPTP) Bali, Denpasar.

FAUZI M et al. 2017. Karakteristik Kimia Kopi Luwak Robusta Artifisial Terfermentasi Oleh Ragi Luwak Dan A-Amilase. Jurnal Penelitian Pascapanen Pertanian 14(3): 144–153.

FIORESI, DB et al. 2021. Mid infrared spectroscopy for comparative analysis of fermented arabica and robusta coffee. Food Control. 121: 1–8.

HAILE M & KANG W H. 2019a. The role of microbes in coffee fermentation and their impact on coffee quality. Journal of Food Quality 2019: 6p.

HAILE M & KANG WH. 2019b. Antioxidant activity, total polyphenol, flavonoid and tannin contents of fermented green coffee beans with selected yeasts. Fermentation 5: 29.

HANIFAH N & KURNIAWATI D. 2013. Pengaruh larutan alkali dan yeast pada proses pembuatan kopi fermentasi. Jurnal Teknologi Kimia Dan Industri 2(2): 162–168.

HATININGSIH S et al. 2018. Microbiological And Physicochemical Changes Of Green Coffee (Coffea Arabica) Fermentation In Kintamani, Bangli, Bali. Scientific Journal of Food Technology 5(2): 123–138.

ICO. 2019. INTERNATIONAL COFFEE ORGANIZATION. Total production by all exporting countries Retrieved 20 Jan 2020, from: https ://www. ico.org/price s/po-production.pdf.

JUMHAWAN U et al. 2013. Selection of discriminant markers for authentication of Asian palm civet coffee (Kopi Luwak): A metabolomics approach. Journal of Agricultural and Food Chemistry 61: 7994–8001.

JUMHAWAN U et al. 2015a. Application of gas chromatography/flame ionization detector-based metabolite fingerprinting for authentication of Asian palm civet coffee (Kopi Luwak). Journal of Bioscience and Bioengineering 120: 555–561.

JUMHAWAN U et al. 2015b. Application of gas chromatography/flame ionization detector-based metabolite fingerprinting for authentication of Asian palm civet coffee (Kopi Luwak). Journal of Bioscience and Bioengineering 120: 555–561.

KADIC E & HEINDEL TJ. 2010. Mixing considerations in stirred tank bioreactors when using fluid property altering microorganisms. In: 3rd Joint US-European Fluids Engineering Summer Meeting and 8th International Conference on Nanochannels, Microchannels, and Minichannels, Proceeding of the ASME. p.859–870.

KIM I et al. 2021. Physicochemical characteristics of Ethiopian *Coffea arabica* cv. Heirloom coffee extracts with various roasting conditions. Food Sci Biotechnol. 30(2):235-244. doi: 10.1007/s10068-020-00865-w.

KITZBERGER CSG et al. 2020. Flavor precursors and sensory attributes of coffee submitted to different post-harvest processing. AIMS Agriculture and Food 5(4), 700-714.https://doi.org/10.3934/agrfood.2020.4.700.

KIYAT WE et al. 2019. Review: Potensi mikrobial selulase, xilanase, dan protease dalam fermentasi kopi luwak (Paradoxurus hermaphroditus) secara in vitro. Jurnal Kimia Sains dan Aplikasi 22(2): 58–66.

LATIEF M et al. 2022. Preliminary Data on the Antibacterial Activity of Coffea Arabica, Coffea Canephora and Coffea Liberica. Pharmacognosy Journal 14(2): 413-424.

LATIEF M et al. 2023. Coffee Improvement by Wet Fermentation Using Lactobacillus plantarum: Sensory Studies, Proximate Analysis, Antioxidants, and Chemical Compounds. Journal of Chemical Engineering and Environment 7(2): 134-148.

LEE LW et al. 2016. Modulation of coffee aroma via the fermentation of green coffee beans with Rhizopus oligosporus: I: Green coffee. Food Chemistry 211:916-924, 2016.

MARDIANA R et al. 2021. Pengaruh Suhu Roasting Terhadap Perubahan Kadar Lemak, Kadar Asam Total, dan Morfologi Mikrostruktural Kopi Robusta. Prosiding

The 12th Industrial Research Workshop and National Seminar. 4-5 Agustus 2021, Bandung. 151-156.
MARTINEZ SJ et al. 2021. Novel stainless steel tanks enhances coffee fermentation quality. Food Research International 139: 109921.
MULLER, A. J et al. 2013. Avaliação da Qualidade Físico-Química de Diferentes Marcas de Café Torrado Solúvel e em Pó Comercializadas na Região do Vale do Taquari (RS). Revista Brasileira de Tecnologia Agroindustrial 7:1004-1012.
MAWARDHI AD & SETIADI D. 2018. Strategi Pemanfaatan Lahan Gambut Melalui Pengembangan Agroforestri Kopi Liberika (Coffea Liberica) (S. Herlinda (Ed.).). UNSRI Press. p.43–51.
MOLDVAER A. 2014. Coffee obsession: flavoursome beans and barista techniques over 100 global recipes. London: Dorling Kindersley Limited.
PAMUNGKAS MT et al. 2021. Pengaruh suhu awal dan lama penyangraian (roasting) terhadap sifat fisik dan kimia pada seduhan kopi Arabika (Coffea arabica L) dari Kabupaten Gayo, Provinsi Aceh. Jurnal AGROTEC 3(2): 1-10.
PANGGABEAN E. 2011. Perbandingan Karakteristik Fisis Kopi Luwak (Civet Coffee) dan Kopi Biasa Jenis Arabika. Jurnal Kimia 2(2): 68-75.
PEREIRA DMGV et al. 2014. Isolation, selection and evaluation of yeasts for use in fermentation of coffee beans by the wet process. International Journal of Food Microbiology 188: 60–66.
PEREIRA DMGV et al. 2019. Exploring the impacts of postharvest processing on the aroma formation of coffee beans—A review. Food Chemistry 272: 441–452.
SAHACHAIRUNGRUENG W et al. 2022. Assessing The Levels of Robusta and Arabica in Roasted Ground Coffee Using Nir Hyperspectral Imaging and FTIR Spectroscopy Foods 11: 1-13.
SARAGIH B et al. 2021. Profile of FTIR (Fourier Transform Infra Red) and comparison of antioxidant activity of coffee with tiwai (*Eleutherine americana* merr). Pap 6th Int Conf Food, Agric Nat Resour. 16: 27-31.
SHARMA R. et al. 2020. Microbial Fermentation and Its Role in Quality Improvement pf Fermented Foods. Fermentation 6(4): 1-20.
SILABAN R et al. 2023. Pengaruh jenis kemasan terhadap karakteristik fisikokimia dan sensori kopi arabika gayo dengan metode pengolahan semi basah. Jurnal Teknologi Pangan dan Hasil Pertanian 18(2):13
SIMATUPANG M et al. 2023. FTIR-ATR fingerprinting of robusta and arabica coffee fraction and its correlation with their antioxidant activity. Jurnal Teknologi dan Industri Pangan 34(1):70-85.
SUHARYONO & BUSYRA B. 2019. Potensi, Kendala Dan Prospek Pengembangan Kopi Liberika: Studi Kasus Petani Kopi Liberika Kelurahan Mekar Jaya Kabupaten Tanjung Jabung Barat Jambi. Jurnal Ilmiah Ilmu Terapan Universitas Jambi 3: 93–99.
TARIGAN IL et al. 2024a. Changes in the Chemical Compound and Sensory Profiles of Liberica Fermentation (*Coffea liberica*) with Cellulolytic Bacteria Alcaligenes sp and Exiguobacterium indicum. Coffee Science, 19, e192258. doi: 10.25186/.v19i.2258.

TARIGAN IL et al. 2024b. Enhancement of Liberica Coffee Quality by Wet Fermentation using Bacillus Subtilis. Alchemy Jurnal Penelitian Kimia 20(2): 162-177.
TAWALI AB et al. 2018. Pengaruh Fermentasi Menggunakan Bakteri Asam Laktat Yoghurt Terhadap Citarasa Kopi Robusta (Coffea Robusta) Canrea Journal: Food Technology, Nutritions, And Culinary Journal 1: 90–97.
USMAN D et al. 2015. Fermentasi Kopi Robusta (*Coffea canephora*) Menggunakan Isolat Bakteri Asam Laktat dari Feces Luwak Dengan Perlakuan Lama Waktu Inkubasi. Jurnal Akademika Biologi 4: 31-40.
WANG N & LIM LT. 2012. Fourier Transform Infrared and Physicochemical Analyses of Roasted Coffee. J Agric Food Chem 60: 5446-5453.
WHELAN BL et al. 2023. Welfare and Management of Civets in Civet Coffee Tourism Plantations. Journal Of Applied Animal Welfare Science 27 (3): 561-574.
WILUJENG AAT & WIKANDARI PR. 2013. The Effect of Fermentation Time of Arabica Coffe (*coffea arabica*) with *Lactobacillus plantarum* B1765 Lactic Acid Bacteria to the Product Qualites. UNESA Journal of Chemistry 2(3): 1-10.
ZAINUDDIN A & TOMINA S. 2021. The Effects of Long Fermentation on Phisical and Chemical Characteristics of Pinogu Coffee. Gorontalo Agriculture Technology Journal 4: 35-43.